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Research Note

Chlorine Inactivation of *Escherichia coli* O157:H7 in Water

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ABSTRACT

Six human isolates of *Escherichia coli* O157:H7 and *E. coli* (ATCC 11229) were used to determine the concentrations of free chlorine and exposure times required for inactivation. Free chlorine concentrations of 0.25, 0.5, 1.0, and 2.0 ppm at 23°C were evaluated, with sampling times at 0, 0.5, 1.0, and 2.0 min. Results revealed that five of six *E. coli* O157:H7 isolates and the *E. coli* control strain were highly susceptible to chlorine, with >7 $\log_{10}$ CFU/ml reduction of each of these strains by 0.25 ppm free chlorine within 1 min. However, comparatively, one of the seven strains was unusually tolerant to chlorine at 23°C for 1 min, with a 4-, 5.5-, 5.8-, and >5.8-log CFU/ml reduction at free chlorine concentrations (ppm) of 0.25, 0.5, 1.0, and 2.0, respectively. Based on these studies most isolates of *E. coli* O157:H7 have no unusual tolerance to chlorine; however, one strain was exceptional in being recovered after 1-min of exposure of 10^7 CFU/ml to 2.0 ppm of free chlorine. This isolate may be a useful reference strain for future studies on chlorine tolerance of *E. coli* O157:H7.

Since 1982, outbreaks of *Escherichia coli* O157:H7 infections have been associated with a variety of foods, including apple cider, raw milk, alfalfa sprouts, undercooked ground beef patties, and beef-related products *(10, 19)*. In addition, many outbreaks associated with drinking or swimming water contaminated with *E. coli* O157:H7 have been reported in the United States *(7, 12, 14, 18),* South Africa *(11),* Finland *(16),* and Scotland *(8)*. In the summer of 2000, contamination by *E. coli* O157:H7 of the municipal water supply of Walkerton, Ontario, Canada resulted in an outbreak of more than 2,000 cases and 6 deaths *(2)*.

During June 1998, 26 children were infected with *E. coli* O157:H7 at a Georgia water park *(5)*. Illness was associated with swimming in a specific pool. In addition, one other outbreak of *E. coli* O157:H7 infection associated with an ostensibly chlorinated pool has been reported *(9)*. The occurrence of these outbreaks has raised questions regarding the efficacy of chlorine for killing *E. coli* O157:H7 in water.

Chlorination is the primary treatment in the United States used to ensure that both drinking and recreation water are free of pathogenic bacteria. A federal minimum criterion of 0.2 ppm of free chlorine has been established for disinfecting drinking water. The objective of this study was to determine the efficacy of different levels of free chlorine to kill different isolates of *E. coli* O157:H7 in water.

MATERIALS AND METHODS

Bacteria. Six strains of *E. coli* O157:H7 isolated from human patients, identified as G, H, I, J, K, L, and a strain of *E. coli* (ATCC 11229) as the control were used. Four of the strains that had the same distinctive pulsed-field gel electrophoretic pattern were isolated from persons who became ill after visiting a Georgia water park in June 1998 *(5)*; the two additional isolates were from sporadic Georgia cases in June and July 1998, in persons whose illnesses were not associated with the water park.

Preparation of bacteria. Each strain of *E. coli* O157:H7 and the *E. coli* control were grown individually at 37°C on nutrient agar (Difco Laboratory, Detroit, Mich.) and transferred at least three times at 24-h intervals before use. Bacteria were harvested from nutrient agar plates by suspension in 0.1 M phosphate buffer, pH 7.4. Cells were washed twice in 0.1 M phosphate buffer three times by sedimenting by centrifugation at 8,000 × *g* for 20 min and suspended in 0.1 M phosphate solution. The bacterial cell suspension was adjusted to an optical density reading at 630 nm of 0.1 (10^8 CFU/ml). Bacterial cell populations were determined by plating in duplicate 0.1-ml serial (1:10) dilutions on tryptic soy agar (Beckton Dickinson, Sparks, Md.) plates.

Water. Deionized, unchlorinated water was filter-sterilized by passing through a 0.22-μm filter (Nalge Nunc international, Rochester, NY).

Chlorine preparations. Standard chlorine solution obtained from Hach Co. (Loveland, Colo.) was freshly diluted for each experiment in deionized water to the required concentration. The concentration of free chlorine in dilute chlorine solutions was determined with a Digital Titrator (Hach Co.) model 16900.

Chlorine sensitivity assay. The protocol of the Association of Official Analytical Chemists was used to determine chlorine sensitivity of the *E. coli* strains. *E. coli* suspension (1 ml) was added to 199 ml of chlorine solution (22 to 23°C) being stirred with a magnetic stir bar in a 500-ml Erlenmeyer flask. At predetermined sampling times, 1.0 ml of the treated bacterial suspension was removed and mixed with 9.0 ml of neutralizing buffer

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TABLE 1. *Inactivation of* E. coli *and* E. coli O157:H7 by chlorine at 23°C

Free chlorine concentration (ppm)	Strain	*E. coli* ($\log_{10}$ CFU/ml) at contact time (s)			
		0	30	60	120
0.25	*E. coli* O157:H7 G	7.5 ± 0.2	4.2 ± 0.6	3.5 ± 0.5	1.7 ± 0.1
	E. coli O157:H7 H	7.6 ± 0.3	—[a]	—	—
	E. coli O157:H7 I	7.4 ± 0.2	—	—	—
	E. coli O157:H7 J	7.3 ± 0.1	—	—	—
	E. coli O157:H7 K	7.6 ± 0.3	—	—	—
	E. coli O157:H7 L	7.5 ± 0.2	<1.7[b]	—	—
	E. coli ATCC 11229	7.4 ± 0.1	2.4 ± 0.5	—	—
0.5	*E. coli* O157:H7 G	7.5 ± 0.2	3.7 ± 0.6	2.0 ± 0.2	<1.7
	E. coli O157:H7 H	7.6 ± 0.3	—	—	—
	E. coli O157:H7 I	7.4 ± 0.2	—	—	—
	E. coli O157:H7 J	7.3 ± 0.1	—	—	—
	E. coli O157:H7 K	7.6 ± 0.3	—	—	—
	E. coli O157:H7 L	7.5 ± 0.2	<1.7	—	—
	E. coli ATCC 11229	7.4 ± 0.1	—	—	—
1.0	*E. coli* O157:H7 G	7.5 ± 0.2	3.4 ± 0.5	1.7 ± 0.1	<1.7
	E. coli O157:H7 H	7.6 ± 0.3	—	—	—
	E. coli O157:H7 I	7.4 ± 0.2	—	—	—
	E. coli O157:H7 J	7.3 ± 0.1	—	—	—
	E. coli O157:H7 K	7.6 ± 0.3	—	—	—
	E. coli O157:H7 L	7.5 ± 0.2	—	—	—
	E. coli ATCC 11229	7.4 ± 0.1	—	—	—
2.0	*E. coli* O157:H7 G	7.5 ± 0.2	2.9 ± 0.2	<1.7	—
	E. coli O157:H7 H	7.6 ± 0.3	—	—	—
	E. coli O157:H7 I	7.4 ± 0.2	—	—	—
	E. coli O157:H7 J	7.3 ± 0.1	—	—	—
	E. coli O157:H7 K	7.6 ± 0.3	—	—	—
	E. coli O157:H7 L	7.5 ± 0.2	—	—	—
	E. coli ATCC 11229	7.4 ± 0.1	—	—	—

[a] —, not detectable by direct plating and an enrichment procedure.
[b] <1.7 log, detectable by an enrichment procedure but not by direct plating.

(Difco). Bacteria were serially (1:10) diluted in 0.1% peptone water and 0.1 ml of each dilution was surface plated on eosin methylene blue agar plates in duplicate. The plates were held at 37°C for 24 h and *E. coli* colonies were enumerated *(3).* At least one colony from each plate was confirmed as *E. coli* O157:H7 by biochemical and immunological methods as we described previously *(21).* A 1.0-ml portion of bacterial suspension from each tube of neutralizing buffer was added to a test tube containing 10 ml of lactose broth and incubated at 37°C for 24 h for enrichment culture of surviving *E. coli.* A loopful of broth was surface plated on each of duplicate eosin methylene blue agar plates that were then held at 37°C for 24 h, and selected colonies typical of *E. coli* were confirmed according to the procedures described above. All experiments were duplicated, and averages of results are reported.

RESULTS AND DISCUSSION

A chlorine concentration of 0.25 ppm killed more than 10^7 CFU/ml of *E. coli* O157:H7 in 30 s, and within 60 s killed more than 10^7 CFU/ml of *E. coli* ATCC 11229 (Table 1). *E. coli* O157:H7 strain G from a sporadic Georgia case not associated with the water park was unusually tolerant, with 50 CFU/ml detected at 120 s of contact time. A similar trend was observed with higher levels of free chlorine, with strain G detected by enrichment culture after treatment with 0.5 and 1.0 ppm for 120 s, and no other strains of *E. coli* O157:H7 or *E. coli* surviving treatment with 0.5 ppm for 60 s or 1.0 ppm for 30 s. Interestingly, strain G was detected by enrichment culture after treatment with 2.0 ppm free chlorine for 60 s. Clearly, *E. coli* O157:H7 strain G has unusual tolerance to free chlorine compared to the other *E. coli* and *E. coli* O157:H7 strains evaluated.

Previous studies have revealed that hypochlorous acid (chlorine) kills *E. coli* by damaging the respiratory and transport processes of the cell membrane *(1, 4).* Lisle et al. *(13)* determined that chlorine inactivated *E. coli* O157:H7 by the same mechanism. Their results suggested that the sensitivity of *E. coli* O157:H7 to chlorine was similar to that of other *E. coli.*

Rice et al. *(17)* determined the effect of low levels of chlorine on seven strains of *E. coli* O157:H7 isolated from cattle from geographically distinct areas (Florida, Idaho, Illinois, Missouri, Texas, Washington, and Wisconsin) and on four strains of *E. coli.* Their results revealed that the *E. coli* O157:H7 isolates were sensitive to 1.1 ppm free chlorine with inactivation of 4 $\log_{10}$ CFU/ml within 1 min. Similar results were obtained for the wild-type *E. coli* isolates. We obtained similar results for six (five *E. coli* O157:H7 and one *E. coli*) strains we evaluated; however, one strain of *E. coli* O157:H7 was exceptionally tolerant, with survivors detected after exposure to 1 and 2 ppm free chlorine for 2 and 1 min, respectively. Hence, there appears to be strain

differences in tolerance to chlorine among *E. coli* O157:H7 isolates.

A survey of disinfection practices of municipal water in the United States revealed that water had a median level of chlorine residual of 1.1 ppm and a median exposure time of 45 min in the distribution system before the point of first use *(20). E. coli* O157:H7 at this level of chlorination and contact time is unlikely to survive conventional water treatment practices in the United States, even for the strain identified in this study to be unusually tolerant to chlorine. However, this chlorine-tolerant strain of *E. coli* O157:H7 may be a concern for recreational waters, such as swimming pool water for which there are many influences that reduce the chlorine concentration and time of exposure to effective concentrations of chlorine. Factors affecting chlorine levels of swimming pool water include sunlight, plant tissues (e.g., leaves and twigs), suntan lotion, urine, fecal matter, and body sweat. Free chlorine can readily react with organic materials in water thereby neutralizing its antimicrobial activity *(6).*

Mokgatla et al. *(15)* isolated from a poultry abattoir a strain of *Salmonella* that was unusually tolerant to hypochlorous acid and could grow in the presence of a chlorine concentration considered to be antibacterial. Hence, there is evidence of the occurrence of a relatively chlorine-tolerant foodborne pathogen, indicating that enteric bacteria have the capacity to develop exceptional tolerance to chlorine. Additional research is needed to determine the prevalence of moderate and highly chlorine-resistant strains of enteric pathogens to enable assessment of the risk of exposure to such pathogens in potable water.

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